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Presence of the *Eucalyptus* gall wasp *Ophelimus maskelli* and its parasitoid *Closterocerus chamaeleon* in Portugal: First record, geographic distribution and host preference

Manuela Branco · Conceição Boavida · Nicolas Durand · José Carlos Franco · Zvi Mendel

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Abstract The *Eucalyptus* gall wasp *Ophelimus maskelli* (Hymenoptera: Eulophidae) and its parasitoid *Closterocerus chamaeleon* (Hymenoptera: Eulophidae) were observed for the first time in Portugal, in 2006 and 2007, respectively. Data on the distribution of *O. maskelli* in Portugal, differences in the susceptibility of two host species, *Eucalyptus globulus* and *Eucalyptus camaldulensis*, and parasitism by *C. chamaeleon* are given.

M. Branco · N. Durand · J. C. Franco
Centro de Estudos Florestais, Instituto Superior Agronomia, Universidade Técnica de Lisboa,
Tapada da Ajuda,
1349-017 Lisbon, Portugal

C. Boavida
Instituto Nacional de Recursos Biológicos,
Ministério da Agricultura,
Desenvolvimento Rural e Pescas,
Edifício 1, Tapada da Ajuda,
1349-017 Lisbon, Portugal
e-mail: mcboavida@dgpc.min-agricultura.pt

Z. Mendel
Department of Entomology, ARO, The Volcani Center,
Bet Dagan 50250, Israel
e-mail: zmendel@volcani.agri.gov.il

M. Branco (✉)
Departamento de Engenharia Florestal,
Instituto Superior Agronomia,
Universidade Técnica de Lisboa,
Tapada da Ajuda,
1349-017 Lisbon, Portugal
e-mail: mrbranco@isa.utl.pt

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Ophelimus maskelli (Ashmead) (Hymenoptera: Eulophidae) is a gall wasp causing damage on *Eucalyptus* species. It induces numerous small pimple-like, nearly round galls visible on both sides of the leaf (Protasov et al. 2007b). In Israel its population produces three generations per year, between spring and the end of fall, the duration of the entire gall development being 80–90 days (Protasov et al. 2007b). In Europe, the wasp was first recorded, in Italy in 2000, misidentified as *O. eucalypti* (Arzone and Alma 2000; Viggiani and Nicotina 2001). Later, it was detected in the south of Spain in 2003 (Sánchez 2003), in the northeast of Spain in 2004 (Pujade-Villar and Riba-Flinch 2004), and in the south of France in 2005 (European and Mediterranean Plant Protection Organization 2006). No signs of its presence were observed during intensive surveys carried out in 2003 and 2004 in Portugal in order to detect *Eucalyptus* gall wasps (Branco et al. 2006) when the only wasp species found was *Leptocybe invasa* Fisher & LaSalle (Hymenoptera: Eulophidae). *Ophelimus maskelli* was first detected in April 2006, near Lisbon, on *Eucalyptus camaldulensis* (M. Branco, unpublished data).

Eucalyptus globulus and *E. camaldulensis* are the major *Eucalyptus* species in the Iberian Peninsula; other species are seldom found. The main *Eucalyptus* forests are based on *E. globulus*. *Eucalyptus camaldulensis* is mostly used in leisure, roadside and urban areas. Due to the importance of these two *Eucalyptus* species, a monitoring program was designed to survey the distribution of *O. maskelli* in Portugal, and to confirm the susceptibility of *E. globulus* and *E. camaldulensis* to this wasp, as reported by Protasov et al. (2007a) in Israel. Unexpectedly, during this survey, the parasitoid *Closterocerus chamaelon* Girault (Hymenoptera: Eulophidae) was found among the wasps emerging from *O. maskelli*-infested leaves collected in Lisbon from *E. camaldulensis*. The parasitoid was identified according to Protasov et al. (2007a). This species was first collected in Australia (Mendel et al. 2007; Protasov et al. 2007a), and imported and released in Israel, in 2005 and 2006 (Mendel et al. 2007; Protasov et al. 2007a), and to Italy in 2006 (Laudonia et al. 2006), for biological control of *O. maskelli*.

In this paper, the geographical distribution of *O. maskelli* is reported together with the spontaneous occurrence of *C. chamaeleon* in Portugal. Information on differences in host plant susceptibility and preliminary results on parasitoid activity are presented.

In spring 2007, a countrywide survey of *E. globulus* and *E. camaldulensis* trees was carried out on 23 locations to detect the presence of *O. maskelli*. At each location five to ten trees were inspected by observing at least five branches on each and the presence of the typical galls induced by the wasp was recorded.

In September 2007, *O. maskelli*-infested leaves were collected on two occasions in an *E. camaldulensis* coppice, in Lisbon, in order to develop a rearing procedure in the laboratory. The leaves were chosen among those having fully developed galls and left in containers for one month, before counting the emerged wasps.

In October/November 2007, samples were collected from infested trees of both *E. globulus* and *E. camaldulensis*, in two locations, Lisbon and Peninsula of Setúbal, about 60 km south of Lisbon, to assess differences in susceptibility between host plant species. Seven *E. camaldulensis* and eight *E. globulus* trees were randomly sampled from the same stand in each location. Six 40–50-cm-long branches were randomly collected per sampled tree, using telescopic pruning scissors, and placed in separate labeled plastic bags. Each sample was observed in the laboratory to determine its infestation level using the following notation: class 0—leaves with no *O. maskelli* galls; class 1—leaves with one to 40 galls/leaf; class 2—leaves with more than 40 galls/leaf. An ANOVA was used to test the effects of *Eucalyptus* species on the percentage of leaves on each infestation class applied by General Linear Model procedure (SPSS version 15 software). Arcsine transformation was used to stabilize variance. Separate analyses were performed for each infestation class. Species effects were tested by simple contrast.

The susceptibility of the two *Eucalyptus* species to *O. maskelli* was evaluated also based on gall size. A sample of 30 galls per each tree species was collected from different leaves and their diameter was measured. Differences between plant species were compared by using a *t*-test on untransformed data.

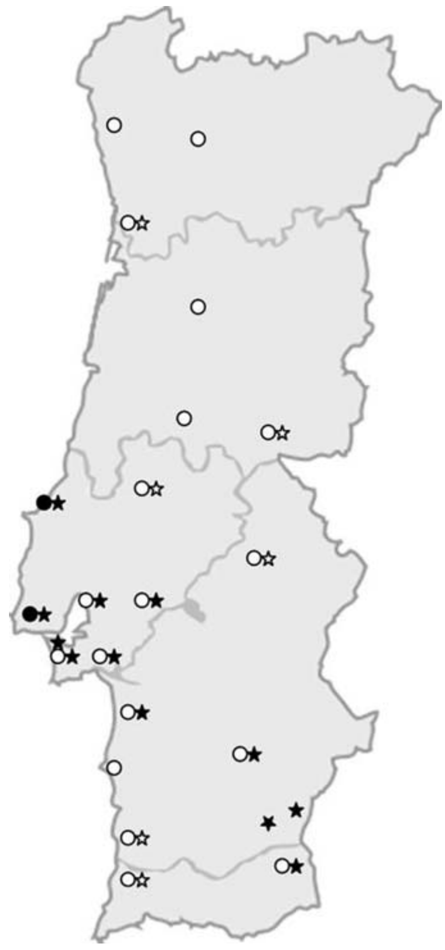

Fig. 1 Distribution of *Ophelimus maskelli* in Portugal in 2007, based on the presence of typical leaf galls in two *Eucalyptus* species: (*closed stars*) *E. camaldulensis* with galls; (*open stars*) *E. camaldulensis* with no signs of galls; (*closed dots*) *E. globulus* with galls; (*open dots*) *E. globulus* with no signs of galls

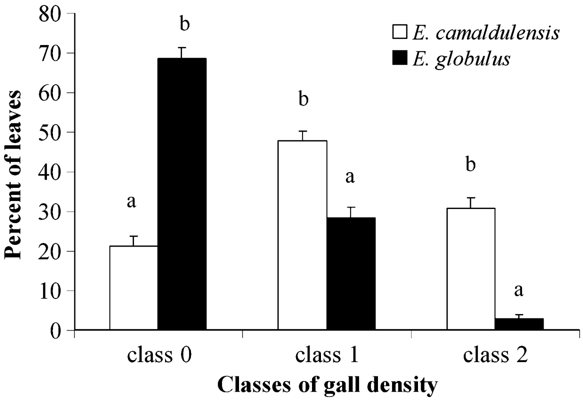

Fig. 2 Percentage of galled leaves of *Eucalyptus camaldulensis* and *Eucalyptus globulus* by *Ophelimus maskelli* according to three classes of leaf gall density: 0—no galls, 1—fewer than 40 galls per leaf, 2—more than 40 galls per leaf. *Bars capped with the same letter* in each class are not significantly different ($P<0.001$)

The emergences of *O. maskelli* and its parasitoid *C. chamaeleon* were assessed in infested leaf samples of *E. camaldulensis* collected in the middle part of the same branches used to assess the infestation levels of *O. maskelli* in the two locations referred to before (October/November 2007). No samples were collected from *E. globulus* because of its very low level of infestation by *O. maskelli*. In the laboratory the leaves were examined and the number of galls without wasp emergence holes was counted per leaf and considered as the number of suitable galls available for parasitisation by *C. chamaeleon* close to the sampling date. Galls with emergence hole were not considered since it was not possible to know whether it was the galler or the parasitoid that emerged from them. The samples were kept in containers in the laboratory, at room conditions for one month and the emerged wasps were collected and counted every week.

The survey conducted in Portugal in 2007 showed that *O. maskelli* was present in 12 out of 23 sites, mainly in two areas, the South, near the border with Spain, and the Centre, near Lisbon (Fig. 1). During this field survey, the gall wasp was mainly observed in *E. camaldulensis* (Fig. 1). In agreement, the percentage of infested leaves (classes 1 and 2) in *E. camaldulensis* was significantly higher ($F_{1,178}=20.9$; $p<0.001$ and $F_{1,178}=103.5$; $p<0.001$, respectively) than in *E. globulus* (Fig. 2) while the percentage of leaves with no galls was significantly higher ($F_{1,178}=146.6$, $p<0.001$) in *E. globulus* (about 70%), compared to *E. camaldulensis* (about 20%). Gall mean diameter (±SE) was significantly smaller in *E. globulus* (0.88±0.037 mm), and most of these galls showed no wasp emergence, in comparison with *E. camaldulensis* (1.42±0.051 mm; $t=8.58$; $df=58$; $p<0.001$).

Closterocerus chamaeleon was found in Portugal for the first time in the beginning of September 2007 in leaf samples infested with *O. maskelli* galls collected from *E. camaldulensis* showing 51.5–63.5% of parasitism (Table 1). The mean number of galls/leaf (±SE) was 155.3±14.9. Later, in October/November 2007, almost only the parasitoid wasps emerged from the galls collected in the survey and both *O. maskelli* and C. *chamaeleon* emerged in very few numbers from the samples (Table 1).

According to the survey reported previously (Branco et al. 2006), *O. maskelli* invaded Portugal between 2004 and 2006, probably from the south of Spain, where it was observed in 2003 (Sánchez 2003). However, no previous reports exist confirming the presence of *C. chamaeleon* in the western Mediterranean Region and Napoli (in Italy), about 2,700 km from Lisbon, seems to be the nearest area where this parasitoid had been released (Laudonia et al. 2006). *Closterocerus chamaeleon* has several biological traits that favor population increase and spread to new zones, such as thelytoky, high fecundity, short generation time, and in particular the relatively high

Table 1 Cumulative emergences of *Ophelimus maskelli* and its parasitoid, *Closterocerus chamaeleon*, from leaves of *Eucalyptus camaldulensis* collected in 2007 in Lisbon and Peninsula of Setubal, Portugal

Date and location	Number of leaves	Number of galls	*O. maskelli*	*C. chamaeleon*	Percent galls with emergence of *C. chamaeleon*
September, Lisbon	10	1,104	398	701	63.5
	16	2,588	1,245	1,333	51.5
October, Lisbon	28	4,349	0	1,838	42.3
November, Setubal	40	8,453	55	810	9.6

adult longevity coupled with its very small size, which favors dispersal by wind (Protasov et al. 2007a). In Israel, the parasitoid spread 120 km in one year (Mendel et al. 2007; Protasov et al. 2007a). In Turkey, a population of *C. chamaeleon* found in January 2007 is thought to have originated from individuals dispersed from the population released in Israel, approximately 1,500 km away (Doganlar and Mendel 2007). The greatest dispersal distances traveled by released parasitoids have been reported as 150 km per year (Godfray 1993). However, spread rates may be much higher, reaching 100 to 300 km per day, when transport by prevailing winds occurs (Farrow 1981). Hence it might be possible that this parasitoid is established in southwestern Mediterranean taking advantage of the high population densities of its host on *E. camaldulensis* in the area.

Although *E. globulus* is a suitable host-plant species for *O. maskelli* as indicated by Protasov et al. (2007b), the present results provide evidence that its susceptibility is much lower compared to *E. camaldulensis*. This is to be predicted by differences in gall density and gall size, which were both significantly lower in *E. globulus*. In particular, gall size is an important predictor of galler survival and therefore of its fitness (Stone and Schönrogge 2003). According to the present finding, host-plant genotype may have a significant impact on gall size for this galler–host plant system. The data also predict significant differences in gall density between trees within species, evidencing intraspecific variability to *O. maskelli* within these two *Eucalyptus* species.

Closterocerus chamaeleon was reared from all leaf samples infested with *O. maskelli*. The mean percentage of parasitoid emergences from *O. maskelli* galls in collected leaf samples varied between 63.5% in September and 9.6% in November. This value refers to emergences observed during one month and may reflect successful parasitization that occurred just before the collection of samples. In the beginning of September, when *C. chamaeleon* was first collected, the host-to-parasitoid ratio was close to 1:1. Later on, this ratio decreased markedly in favor of the parasitoid, since very few emergences of the gall wasp occurred. This pattern indicates that the parasitoid may remain active in the field for longer periods than the host species. Host and parasitoid phenologies need to be investigated throughout the year, in order to understand the distributions observed in field samples.

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